CLAIMS

1. A method of determining an analyte by means of luminescence assay in which

(i) the donor species is provided as, in or adsorbed to a solid phase and is an up-conversion medium capable of effecting an energy transition to an excited state by absorption of electromagnetic radiation having an energy less than that of said transition, and

(ii) the acceptor species is bound or is capable of being bound directly or indirectly to the surface of said solid phase and is capable of being excited by energy transfer from the donor species,

the method comprising the steps of

(iii) irradiating the donor species with said electromagnetic radiation, to excite the donor species to the excited state, and

(iv) detecting luminescence in at least one spectral region characteristic of the emission of the donor species and/or the acceptor species provided that the excitation of the acceptor species to an excited state capable of luminescence in a defined spectral region of the acceptor species does not occur by absorption of a single quantum of the radiation used to excite the donor species

characterised in that the analyte causes a change in the excitation condition of at least one of the donor species and an acceptor species as a result of the acceptor species being bound to or released from the solid phase and said change is monitored to determine the analyte

2. A method as claimed in claim 1 wherein the transfer of energy from the donor to the acceptor is detected by decrease in luminescence efficiency of the donor.

3. A method as claimed in claim 1 wherein the acceptor is capable of luminescence subsequent to the said transfer of energy and wherein the luminescence of the acceptor is used to detect the bound species.

4. A method as claimed in claim 1 where the up-conversion medium is excited by simultaneous absorption of two or more photons of the same or different energy.

5. A method as claimed in claim 1 where the up-conversion medium is excited by sequential absorption of two or more photons of the same or different energy.

6. A method as claimed in claim 1 wherein the medium is an up-conversion phosphor designed to absorb long wavelength radiation and to emit light at shorter wavelength.

7. A method as claimed in claim 1 wherein the up-conversion medium requires a priming dose of energy to excite species therein to metastable levels which are required for up-conversion processes to be possible.

8. A method as claimed in claim 7 wherein the up-conversion medium is an electron-trapping phosphor.

9. A method as claimed in claim 1 wherein the up-conversion medium relies on excitation of an organic molecule to an excited state which subsequently relaxes to a metastable level of longer lifetime than the original state and wherein absorption of a second photon promotes re-excitation of the metastable state to another state of higher energy which is capable of luminescence or transfer of energy to an acceptor, or of intersystem crossing giving rise to an excited state of different spin multiplicity that is itself capable of luminescence or transfer of energy to an acceptor.

10. A method as claimed in claim 9 wherein the metastable state is the triplet state of an organic molecule.

11. A method as claimed in claim 1 wherein the up-conversion process is based on the excitation of lanthanide ions in an appropriate matrix.

12. A method as claimed in claim 1 wherein the exciting radiation is absorbed by one or more species within the medium and subsequently transferred to one or more other species within the medium resulting in excitation of the said other species which provides the donor species.

13. A method as claimed in claim 12 wherein the primary absorbing species is the ytterbium ion.

14. A method as claimed in claim 12 wherein the absorbed energy is ultimately transferred within the medium to ions of erbium thulium or other luminescent lanthanide which provides the donor species.

15. A method as claimed in claim 1 wherein the excited species is able to delocalise its excitation by internal transfer of energy between similar sites within the medium.

16. A method as claimed in claim 15 such that delocalisation of excitation results in transfer of energy from sites within the bulk of the medium to sites at or near the surface of the medium which are subsequently able to transfer energy to the bound species.

17. A method as claimed in claim 1 wherein the solid phase is a glassy matrix, e.g. as produced by a sol-gel process.

18. A method as claimed in claim 1 wherein the solid phase is a crystalline matrix.

19. A method as claimed in claim 1 wherein the solid phase is an organic or inorganic polymer.

20. A method as claimed in claim 1 wherein the analyte causes a change in the extent of binding between the solid phase of the donor species and the acceptor species.

21. A method as claimed in claim 20 wherein the assay is conducted in a format in which the analyte causes an increase in the degree of binding of the acceptor to the donor.

22. A method as claimed in claim 20 wherein the action of an enzyme or other catalytic species is monitored by activation of coupling or uncoupling between donor and acceptor either by catalysing formation or cleavage of a linkage between them or by unmasking of protected groups on either or both of the donor and acceptor species, resulting in formation or dissociation of a complex between the said species or formation or cleavage of a chemical bond between them.

23. A method as claimed in claim 20 wherein the assay is conducted in a format in which the analyte causes a decrease in binding of the acceptor to the donor.

24. A method as claimed in claim 20 wherein the surface of the solid phase is so modified as to facilitate binding of the analyte and/or to protect the solid phase from interaction with or dissolution in an aqueous medium.

25. A method as claimed in claim 24 wherein the surface of the solid phase has antibodies, lectins oligonucleotides or other recognition ligands bound to it.

26. A method as claimed in claim 1 wherein the analyte is one which affects the acceptance properties (for energy transfer from the donor species) of a moiety bound to the solid phase of the donor species, and/or which affects the ability of the bound moiety to emit luminescence consequent on excitation by said energy transfer.

27. A method as claimed in claim 26 wherein the assay is used to monitor the formation or cleavage of a bond linking a quenching species or an enhancer of emission to the solid phase or to a luminescent moiety bound thereto.

28. A method as claimed in claim 26 wherein the moiety is one which is converted by the analyte to a luminescent species capable of accepting energy from the excited donor species.

29. A method as claimed in claim 26 wherein the moiety is a pre-existing luminescent label whereof luminescence is quenched by the action of the analyte.

30. A method as claimed in claim 26 wherein the moiety is one which is able to change colour as a consequence of the action of the analyte.

31. A method as in claim 1 wherein the detector is a semiconductor diode.

32. A method as claimed in claim 1 wherein the detector is a linear charge-coupled device or MOS sensor.

33. A method as claimed in claim 1 wherein the detector is an imaging detector.

34. A method as claimed in claim 33 wherein the detector uses an image intensifier or imaging single photon detector.

35. A method as claimed in claim 33 wherein the detector is a CCD camera, or other solid state imaging device.

36. A method as claimed in claim 1 wherein the source of long wavelength radiation is a semiconductor light-emitting diode or laser.

37. A method as claimed in claim 1 wherein the exciting radiation is pulsed or modulated in intensity.

38. A method as claimed in claim 37 wherein gated detection is used to confine detected signals to one or more defined time periods relative to the excitation.

39. A method as claimed in claim 37 wherein the detection system uses a periodically switched, gated or modulated detector to implement lock-in detection or similar correlated detection schemes.

40. A method as claimed in claim 37 wherein the detector is an imaging detector which is gated or modulated in sensitivity.

41. A method as claimed in claim 37 wherein the detector is designed to implement phase-sensitive detection in conjunction with a periodic excitation source.

42. A method as claimed in claim 37 wherein the detector is an interline CCD camera equipped with a charge drain capable of periodically resetting the camera, but wherein charge can be preserved and integrated by transfer to storage registers prior to the drain operation periods for subsequent readout at a later time.

43. A method as claimed in claim 37 wherein the detector is a camera equipped with a fast optical shutter.

44. A method as claimed in claim 1 wherein the observed region of the sample for analysis is illuminated by one or more focused excitation sources of long wavelength photons, such that the efficiency of excitation of the up-conversion medium is markedly greater at or near the focal plane than otherwise as a consequence of a non-linear relationship between photon density and excitation efficiency.

45. A method as claimed in claim 44 wherein two excitation sources of different wavelength are used and the excitation efficiency is proportional to the product of the first or higher powers of the photon densities of each individual excitation source.

46. A method as claimed in claim 1 wherein the up-conversion medium gives rise to two or more luminescence emission peaks which are differently affected by binding of an energy acceptor and where measurements of emission at two or more wavelengths characteristic of these peaks are combined by ratio or otherwise to effect a measurement of the analyte.

47. A method of detection of luminescence in which a luminescent species of interest is excited by a process of simultaneous or sequential absorption of at least two photons from at least one photon source, wherein the or each photon source is modulated at one or more frequencies, and luminescence measurements are made at a frequency or frequencies different from the or each modulation frequency, the or each frequency at which measurements are made being a function of the or each modulation frequency by virtue of the excitation process.

48. A method according to claim 47, wherein the species is excited by photons from a single source which is modulated at a fixed frequency, and measurements are made at a frequency which is a harmonic of the fixed frequency.

49. A method according to claim 47, wherein the species is excited by photons from at least two sources which are modulated at different frequencies, and measurements are made at a frequency which is a function of a linear combination of the different frequencies.

50. A method according to claim 47, wherein the species is excited by photons from a single source which is modulated with a composite waveform comprising the sum of two or more different frequencies, and measurements are made at a frequency which is a function of a linear combination of the different frequencies.

51. A method according to claim 49 wherein the species of interest has a luminescence decay time and the frequencies are selected such that emissions excited by the or each source are not able to reproduce the modulation frequencies but are able to reproduce frequency components corresponding to the difference between the

or at least two of the modulation frequencies, and measurements are made at the frequency of said components.

52. A method according to claim 51, wherein the presence of an interfering species capable of luminescence as the result of multiphotonic excitation processes, but with a shorter luminescence lifetime than the species of interest, is detected by measurement at one or more frequencies selected such that a signal from the species of interest will suffer substantial attenuation by virtue of demodulation while a signal from the interfering species will not suffer substantial attenuation.

53. A method according to claim 52 wherein measurements are made at frequencies that are the sum and difference of the modulation frequencies and, a function of the result of the measurement at the sum frequency is subtracted from a function of the result of the measurement at the difference frequency to suppress contributions from short-lived emissions.

54. A method according to claim 47, wherein the luminescent species is a complex, cryptate or similar moiety comprising one or more organic molecules in close association with one or more metal ions, and wherein the emission originates from electronically excited states of one or more of the metal ions, either directly or by means of energy transfer to another species capable of luminescence.

55. A method according to claim 54, wherein the energy required to excite the metal ion species is transferred thereto from excited organic species in close proximity.

56. An apparatus for detecting luminescence in which a luminescent species is excited by a process of simultaneous or sequential absorption of at least two photons, comprising at least one photon source, means for modulating the or each photon source at a pre-determined frequency, means for detecting emissions at a frequency which is different from the or each modulation frequency but which is a function of the or each modulation frequency by virtue of the excitation process.

57. A method as claimed in claim 1 wherein the solid phase is provided with a continuous, discontinuous or partial coating of a metal for enhancing transfer of energy form the upconversion medium to the acceptor species.

58. A method as claimed in claim 57 wherein analyte recognition molecules are provided and are capable of directly or indirectly binding the acceptor species in close or immediate proximity to the metal.

59. A method as claimed in claim 58 wherein the metal coating is discontinuous and the analyte recognition molecules are bonded to the solid phase at discontinuities in the coating.

60. A composition comprising

(i) a donor species provided as, in or adsorbed to a solid phase and being on upconversion medium capable of effecting an energy transition to an excited state by absorption of electromagnetic radiation having an energy less than that of said transition,

(ii) a continuous, discontinuous or partial metal coating provided on the surface of said phase,

(iii) a first binding agent which is immobilised on said surface or on said metal and which is capable of binding directly or indirectly to a complementary second binding agent,

(iv) a said second binding agent labelled with an acceptor species capable of being excited by energy from the donor species *via* the intermediary of the metal, the acceptor species being such that the excitation thereof to an excited state capable of luminescence in a defined spectral region of the acceptor species does not occur by absorption of a single quantum of radiation used to excite the donor species.

61. A microarray analytical device comprising a plurality of spatially distinct microscopic regions each comprised of binding ligands wherein said ligands are provided on or in close proximity to the surface of an associated metal coating, said device further comprising donor species provided as, in or adsorbed to a solid phase and being an upconversion medium capable of effecting an energy transition to an excited state by absorption of electromagnetic radiation having an energy less than that of said excited state,, said metal coating being provided on the donor species.

62. A microarray as claimed in claim 61 wherein said solid phase is a continuous layer.

63. A device as claimed in claim 62 which is comprised of a plurality of discrete regions of metal coatings each associated with binding ligands, to form said spatially distinct microscopic regions.

64. A device as claimed in claim 62 wherein the metal coating is provided as a continuous layer on said solid phase and said binding ligands are provided on or in close proximity to said continuous metal coating.

65. A method of determining an analyte by means of a luminescence assay comprising the steps of

(i) incubating a microarray as claimed in claim 61 with a sample including an acceptor species that is bound or is capable of being bound directly or indirectly to the surface of said solid phase and is capable of being excited by energy transfer from the donor species,

(ii) irradiating the donor species with said electromagnetic radiation, to excite the donor species to the excited state, and

(iii) detecting luminescence is detected in at least one spectral region characteristic of the emission of the donor species and/or the acceptor species provided that the excitation of the acceptor species to an excited state capable of luminescence in a defined spectral region of the acceptor species does not occur by absorption of a single quantum of the radiation used to excite the donor species

characterised in that the analyte causes a change in the excitation condition of at least one of the donor species and an acceptor species as a result of the acceptor species being bound to or released from the solid phase and said change in monitored to determine the analyte.

66. A method as claimed in claim 65 which is for detecting complementary binding interactions between biomolecules.

67. Apparatus as comprising

(i) a microarray as claimed in claim 61

(ii) a continuous or pulsed source of excitation of a wavelength that can excite upconverted radiation from the microarray,

(iii) means to direct the said excitation to the surface of the microarray,

(iv) optical means to isolate emission in a wavelength range characteristic of the photoluminescence of said bound species while rejecting substantially all of the exciting radiation and of the upconverted emission from the microarray, and

(v) means to detect the characteristic photoluminescence emission of the bound species.